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L56: Entry 1 of 1 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277375 B1
TITLE: Immunoglobulin-like domains with increased half-lives

US PATENT NO. (1):
6277375

Abstract Text (1):
Disclosed are recombinant vectors encoding immunoglobulin-like domains and portions thereof, such as antibody Fc-hinge fragments, subfragments and mutant domains with extended biological half lives. Methods of producing large quantities of such domains, heterodimers, and fusion proteins following expression by host cells are also reported. Described are antibody Fc and Fc-hinge domains, which have the same in vivo stability as intact antibodies; and domains engineered to have increased in vivo half lives. These DNA constructs and protein domains will be useful as templates for in vitro mutagenesis and high resolution structural studies; for immunization and vaccination; and for the production of recombinant antibodies or chimeric proteins with increased stability and longevity for therapeutic and diagnostic uses.

Brief Summary Text (11):
The present invention seeks to overcome deficiencies in the art by providing functional proteins, antibodies or other agents that have an increased serum half-life through the interaction with Fc receptor (FcRn). These functional agents include any molecule that binds to FcRn in a pH dependent way such that binding affinity is strong at about pH 6 to about pH 6.5 relative to binding at pH 7.4. Physiologically, this allows the agent to be salvaged by FcRn at lower pH and released into the essentially neutral pH environment of the serum. The present disclosure includes protein and peptide compositions having altered serum half-lives relative to IgG, methods of making such proteins or peptides, either starting with a known sequence or by screening random sequences, and methods of screening unknown candidate agents for pH dependent FcRn binding. In addition, disclosed herein are methods of making an agent with altered serum half-life by conjugating or otherwise binding of that agent to a moiety identified as having an increased serum half-life through its interaction with FcRn. Such agents would include, but are not limited to antibodies, fragments of antibodies, hormones, receptor ligands, immunotoxins, therapeutic drugs of any kind, T-cell receptor binding antigens and any other agent that may be bound to the increased serum half life moieties of the present invention.

Brief Summary Text (17):
The production of Fc-hinge domains with longer in vivo half lives is an advantageous development in that it further delineates the site for the control of IgG1 catabolism to a specific region of the Fc-hinge fragment, and in practical terms, it has several important applications. It allows the design and construction of antibody molecules, domains, or fragments, such as bivalent Fab fragments, with longer half lives. These would be generally useful in that the slower biological clearance times would result in fewer administrations of any antibody or vaccine such that fewer "booster" vaccinations may be required. Furthermore, these molecules with longer half lives can be used to tag other therapeutic molecules, such as vaccine molecules. The catabolic site delineated in this invention is distinct from the ADCC and complement fixing sites. This is important as antibodies may be produced which are completely functional and which have longer half lives. Other important uses include, for example, antibody-based systemic drug delivery, the creation of immunotoxins with longer lives or even antibody-based immunotherapy for chronic illnesses or conditions such as hay fever or other allergic reactions, or treatment of T-cell mediated autoimmune disorders by anti-T-cell receptor antibodies or T-cell antigens.

Brief Summary Text (20):
In certain other embodiments, the present invention contemplates the creation of recombinant molecules, particularly antibody constructs, including vaccines and immunotoxins, with increased in vivo half lives. Longevity of recombinant molecules is often needed, and several protocols would benefit from the design of a molecule which would be more slowly removed from circulation after exerting its designed action. This may include, for example, antibodies administered for the purpose of scavenging pathogens, toxins or substances causing biological imbalances and thereby preventing them from harming the body; and antibodies designed to provide long-term, systemic delivery of immunotherapeutic drugs and vaccines.

Brief Summary Text (26):
Other DNA segments may also be included linked to the immunoglobulin-like domains described. For example, one or more recombinant antibody variable domains of varying specificities may be linked to one or more antibody constant domains, immunoglobulin constant domains, or even other proteins, such as bacteriophage coat protein genes, hormones or antigens, including T-cell receptor antigens. The antibody constant domains of the present invention may also be combined with another immunoglobulin domain, or indeed, with any other protein. The immunoglobulin constant domains may be variously expressed as a single domain, such as a CH3 domain; or in combination with one, two, three or more domains, such as, for example, as a CH2-hinge domain, an Fc domain, or an entire Fc-hinge domain. In particular embodiments, discussed in more detail below, Fc or Fc-hinge domains may be linked to any protein to produce a recombinant fusion with enhanced biological stability, or certain mutants may be employed to create antibodies or fusion proteins with increased half lives.

Brief Summary Text (53):
In general, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or receptor sites. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a protein with like or even countervailing properties (e.g., antagonistic v. agonistic). It is thus contemplated that various changes may be made in the coding sequences of immunoglobulin-like domains without appreciable loss of the biological utility or activity of the encoded protein. It may even be possible to change particular residues in such domains to enhance their biological utility or to increase their interactive capability, for example, by increasing the binding affinity of Fc for RcRn.

Brief Summary Text (63):
In certain broad aspects, the invention may be described as a method of increasing the serum half-life of an agent comprising conjugating said agent to a mutant IgG or IgG Fc hinge fragment having an increased serum half life as described above. Preferred agents include, but are not limited to a therapeutic drug, an antigen binding polypeptide, an antigen or a receptor binding ligand, or even a T-cell receptor binding ligand, or a T-cell receptor domain.

Detailed Description Text (8):
Apart from antibodies and the T cell receptor, among the best characterized proteins which contain immunoglobulin-like domains are the MHC molecules and the CD4 and CD8 glycoproteins. There are two main classes of MHC (major histocompatibility complex) molecules, class I and II, each consisting of a set of cell-surface glycoproteins. Both classes of MHC glycoproteins are heterodimers with homologous overall structures, the amino-terminal domains of which are thought to be specialized for binding antigen for presentation to T cells. But FcRn, an MHC class I homolog, has a distinct function i.e. the regulation of serum IgG levels.

Detailed Description Text (10):
In common with class I MHC molecules, class II MHC molecules are heterodimers with two conserved immunoglobulin like domains close to the membrane and two polymorphic (variable) amino-terminal domains farthest from the membrane. In these molecules, however, both chains span the membrane. There is strong evidence that the polymorphic regions of both classes of MHC molecules interact with foreign antigen and that it is the complex of MHC molecule and foreign antigen that is recognized by the T cell receptor.

Detailed Description Text (74):

G: Vaccines

Detailed Description Text (75):
The present invention contemplates vaccines for use in both active and passive immunization embodiments. Immunogenic compositions, proposed to be suitable for use as a vaccine, may be prepared most readily directly from engineered antibody Fc fragments. domains and/or peptides prepared in a manner disclosed herein. Preferably the antigenic material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle.

Detailed Description Text (76):
The preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Pat. Nos. 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all incorporated herein by reference. Typically, such vaccines are prepared as injectables. Either as liquid solutions or suspensions: solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccines.

Detailed Description Text (77):
Vaccines may be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides: such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of active ingredient, preferably 25-70%.

Detailed Description Text (78):
The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the peptide) and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Detailed Description Text (79):
The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by subsequent inoculations or other administrations.

Detailed Description Text (80):
The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the size of the host.

Detailed Description Text (81):
Various methods of achieving adjuvant effect for the vaccine includes use of agents such as aluminum hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent

solution in phosphate buffered saline, admixture with synthetic polymers of sugars (Carbopol) used as 0.25 percent solution, aggregation of the protein in the vaccine by heat treatment with temperatures ranging between 70.degree. to 101.degree. C. for 30 second to 2 minute periods respectively. Aggregation by reactivating with pepsin treated (Fab) antibodies to albumin, mixture with bacterial cells such as C. parvum or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed.

Detailed Description Text (82):
In many instances, it will be desirable to have multiple administrations of the vaccine, usually not exceeding six vaccinations, more usually not exceeding four vaccinations and preferably one or more, usually at least about three vaccinations. The vaccinations will normally be at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, will be desirable to maintain protective levels of the antibodies. The course of the immunization may be followed by assays for antibodies for the supernatant antigens. The assays may be performed by labeling with conventional labels, such as radionuclides, enzymes, fluorescers, and the like. These techniques are well known and may be found in a wide variety of patents, such as U.S. Pat. Nos. 3,791,932; 4,174,384 and 3,949,064, as illustrative of these types of assays.

Detailed Description Text (310):
The power of bacteriophage display for the affinity improvement of antibodies for binding to cognate antigen has already been demonstrated. In this study, the system will be used to express mutated Fc fragments and to select for higher affinity variants for binding to FcRn. For this work, milligrams quantities of soluble FcRn (sFcRn) are used and the WT Fc fragment is expressed in functionally active form on the surface of phage.

Detailed Description Text (316):
Two strategies for selection of higher variants from libraries of mutated Fc genes are used. In the first, mutated genes are assembled in pHEN1 (Hoogenboom et al., 1991) and used to transfect E. coli TG1. As indicated above, the leakiness of suppression results in both soluble Fc and phage bound Fc fragments being exported from the recombinant cells, and these should assemble as homodimers on the surface of phage. Phage bearing Fc fragments (`Fc-phage`) are propagated, concentrated by polyethylene glycol precipitation and panned on sFcRn coated Dynabeads/Ni.sup.2+ -NTA-agarose as described previously (Marks et al., 1991; Ward, 1977; Popov et al., 1995). sFcRn are purified from baculovirus infected insect cells as described previously (Popov et al., 1996). Rounds of panning followed by phage propagation should result in enrichment for higher affinity binders. In addition, to select for higher affinity variants, procedures analogous to that described by Winter and colleagues (Hawkins et al., 1992) are used; first, phage are mixed with small amounts of soluble biotinylated sFcRn (<1 .mu.g) such that the antigen is in excess over the phage but at a concentration lower than that of the dissociation constant that is required (7.8 nM; Raghavan et al., 1994). sFcRn bound Fc-phage particles are then used, and Ni.sup.2+ -NTA-agarose added to separate sFcRn bound phage. Second, to select for Fc fragments with lower off rates, Fc-phage particles are preloaded with biotinylated sFcRn and then diluted in to excess unlabeled antigen for variable times prior to addition of streptavidin coated beads as described previously (Hawkins et al., 1992). Alternatively, the selection method used in Example 4 is utilized.

Detailed Description Text (322):
Fc mutants with higher affinity for FcRn are purified from recombinant E. coli cells using the c-myc epitope or polyhistidine peptide as an affinity purification tag as described previously (Ward, 1992; Marks et al., 1991; Popov et al., 1995). The affinities of these Fc fragments for binding to sFcRn are determined using surface plasmon resonance (SPR) and the B Acore (Karlsson et al., 1991). This approach has been used to analyze antibody-antigen interactions (Ward, 1995; Borrebaeck et al., 1992), and Bjorkman and colleagues have characterized the interaction of IgG with rat FcRn using SPR (Raghavan et al., 1994; Popov et al., 1996b).

Detailed Description Text (365):
Bindon, Hale, Waldmann, "Importance of antigen specificity for complement-mediated lysis by monoclonal antibodies," Eur. J. Immunol., 18:1507-1514, 1988.

Detailed Description Text (367):

Borrebaeck, Malmborg, Furebring, Michaelsson, Ward, Danielsson, Ohlin, "Kinetic analysis of recombinant antibody-antigen interaction: relation between structural domains and antigen binding," Bio/technol., 10:697-698, 1992.

Detailed Description Text (426):
Janeway, Conrad, Lerner, Babich, Wettstein, Murphy, "Monoclonal antibodies specific for Ia glycoproteins raised by immunization with activated T cells; possible role of T cell bound antigens as targets of immunoregulatory T cells," J. Immunol. 132:662-667, 1984.

Detailed Description Text (435):
Karlsson, Michaelsson, Mattsson, "Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system," J. Immunol. Methods, 145:229-240, 1991.

Detailed Description Text (464):
Morris, "The receptor hypothesis of protein ingestion," In: Antigen Absorption by the Gut, University Park Press, Baltimore, USA (ed. W.A. Hemmings), pp. 3-22, 1978.

Detailed Description Text (499):
Simister and Mostov, "An Fe receptor structurally related to MHC class I antigens," Eur. J. Immunol., 15:733-738, 1985.

Detailed Description Text (500):
Simister and Mostov, "Cloning and expression of the neonatal rat intestinal Fe receptor, a major histocompatibility complex class I antigen homolog," Cold Spring Harbor Symp. Quant. Biol., LIV, 571-580, 1989b.

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L41: Entry 11 of 17 File: USPT Jan 20, 1998

US-PAT-NO: 5709859
DOCUMENT-IDENTIFIER: US 5709859 A

TITLE: Mixed specificity fusion proteins

DATE-ISSUED: January 20, 1998

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/134.1; 424/136.1, 424/178.1, 435/69.7, 530/387.3, 530/388.22, 530/808, 530/866

ABSTRACT:

Mixed specificity fusion proteins capable of binding to cellular adhesion molecules have been produced. The fusion proteins contain a polypeptide region, such as an IgG constant region, operatively attached to at least two binding regions each of which corresponds to either an extracellular domain of a cell surface receptor for cellular adhesion molecules, or a variable region of an antibody directed to a cellular adhesion molecule.

A method of inhibiting inflammation is a patient is disclosed in which the present fusion proteins are administered to a patient to inhibit the attachment of inflammatory cells to vascular endothelium.

A method of inhibiting metastasis is disclosed in which the present fusion proteins are administered to a patient to inhibit the metastasis of responsive tumor cells.

22 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

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L41: Entry 14 of 17 File: USPT Jun 3, 1997

DOCUMENT-IDENTIFIER: US 5635177 A
TITLE: Protein tyrosine kinase agonist antibodies

DATE FILED (1):
19940404

Abstract Text (1):
Agonist antibodies are disclosed which bind to the extracellular domain of receptor protein tyrosine kinases pTKs, and thereby cause dimerization and activation of the intracellular tyrosine kinase domain thereof. The antibodies are useful for activating their respective receptor and thereby enabling the role of the tyrosine kinase receptor in cell growth and/or differentiation to be studied. Chimeric proteins comprising the extracellular domain of the receptor pTKs and an immunoglobulin constant domain sequence are also disclosed.